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The Significance of Foreign Matter

Seen in the Respiratory Tract After Death

The reconstruction of the mechanism and the cause of death when foreign matter, food or vomit is found in the respiratory tract at autopsy, presents little difficulty for those dogmatists who are content to glimpse the obvious. Indeed, a court may well find their post-mortem observations (especially when unchallenged) commonsensical and plausible and their opinions cogent. However, the recent observations of Gardner[1] stress the need for the greatest caution in making inferences on the basis of such findings, especially when there is no good clinical evidence of the mode of death from eye-witnesses.

Gardner's experiments vindicate the scepticism of those who have long been unimpressed by opinions about the cause of death which depend on the presence of foreign material (even in the depths of the lung), especially when collateral evidence of an asphyxial mode of death is offered by the demonstration of the so-called signs of asphyxia. These signs, as Gordon[2] has shown, are associated with circulatory failure and are present in death from almost any cause. They are entirely non-specific, are certainly not pathognomonic of asphyxia and it is entirely without scientific justification to invest them with an asphyxial significance.

In the course of a study of the aspiration of food and vomit, Gardner experimented with conditions in the cadaver. His results led him to re-emphasize the fact (known at least since 1873) that gastric contents may reach the bronchi after death. In 10 bodies barium sulphate was intro-

1. Gardner, A. M. N. (1958): Quart. J. Med., **27,** 227.
2. Gordon, I. (1944): Brit. Med. J., **2,** 337. (*See also* A.M.A. Arch. Path., **52,** 160).

duced into the stomach through a stomach tube immediately after death. Radiographs of the lungs taken 24–48 hours later revealed barium in the lungs of 7 of the 10 bodies. In some cases it had even reached the alveoli, 'presumably as a result of manipulation of the lungs during their removal.'[3] He concludes:[4]

'The presence of gastric contents in the lungs at autopsy, often ascribed to ante-mortem or agonal aspiration, is not in itself conclusive evidence of such a happening unless, immediately after death, and before moving the body, the trachea has been blocked with an obturator.'

'Furthermore, it may be very difficult even microscopically to differentiate between ante-mortem and post-mortem aspirations, because vital cellular reactions continue in the lungs after clinical death.[5] . . .

(The) diagnosis may also be made erroneously in an attempt to explain morbid anatomical appearances that in fact have resulted from gravitation of gastric contents into the lungs after death.'[6]

Autopsy technique, when the possibility of aspiration of foreign matter is involved, must clearly be modified to exclude the chance of the gravitation of gastric contents into the lungs.

Gardner's observations constitute an important and fundamental contribution to medico-legal pathology which must now be incorporated into the body of forensic knowledge. They serve also to eliminate the expression of irresponsible opinion in a field in which controlled experiment has been all too rare.

3. Gardner, A. M. N. (1958): *Ibid.*, p. 231.
4. Gardner, A. M. N. (1958): *Ibid.*, p. 231.
5. Gardner, A. M. N. (1958): *Ibid.*, p. 237.
6. Gardner, A. M. N. (1958): *Ibid.*, p. 239.

Human and Animal Blood Stains

An Absorption Technique for their Differentiation

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In a previous communication[1] a test which attempted the differentiation of various animal species by means of an absorption technique was described. The principle is that failure of complete absorption of a known antiserum developed against antigens of species A by unindentified antigens of species B, under the particular conditions of the test, is conclusive evidence of differences between the antigens concerned. Agglutination of red blood cells coated with antigens of species A served to reveal the presence of free unabsorbed homologous antibody after the absorption process had been completed. As the antigens involved are proteins, the erythrocytes had to be tanned[2] before they would absorb the species A antigens.

Shortcomings were mentioned in the article referred to and subsequent extensions and modifications of the test, as applied to the differentiation of human and animal blood stains, form the basis of the present communication.

Experimental Materials, Methods, and Results

Buffered Saline. This was prepared by mixing appropriate volumes[3] of M/15 $Na_2 HPO_4$ and M/15 $KH_2 PO_4$ to give solutions with pH values of either 6·24 or 7·38; 8·5 g. NaCl (Analar) was added to every 1,000 ml. of buffer solution. Solutions with the latter pH were used throughout except where the former is specifically indicated.

Sheep Erythrocytes. These were collected in Alsever's solution and stored at 4°C. Fresh cells were obtained at weekly intervals. The cells were washed 3 times in buffered saline before use.

Tannic Acid. A fresh 1:25,000 solution of Tannic Acid (B.D.H. laboratory reagent) in buffered saline was prepared for each batch of tests.

Normal Rabbit Serum (NRS). This was obtained fresh for each batch of experiments and was used unabsorbed.

Anti-Human Globulin Serum. Burroughs Wellcome Coombs' reagent, series K.9278 with an homologous haemagglutination titre of 1:8,192, was used in one test to be described.

Blood Specimens. Whole blood from the following species was used: man, chimpanzee, vervet monkey, chacma baboon, ox, meercat, rat, mouse, rabbit, guinea-pig and fowl.

These specimens were frozen at —10°C. immediately on receipt and maintained in this condition until required. Upon thawing, the haemolysed blood was centrifuged to clear it of any clots. This clear mixture of serum, haemoglobin, etc. will subsequently be referred to as blood.

Blood Stains. These were prepared by depositing drops of blood on pieces of clean linen. Extracts were prepared from these stains by cutting them out, immersing the cloth in 1—2 ml. of buffered saline at 4°C. for 18 hours and then centrifuging to clear.

The stains varied in age from 1 day to 3 months.

The protein concentration (as judged visually by sulphosalicylic acid precipitation[4]) of the 2 antigens used in each absorption test was adjusted to the same order by dilution with buffered saline. The object was to have all the extracts as concentrated as possible. All sera and extracts used (with the exception of NRS) were routinely decomplemented at 56°C. for 30 minutes, and then absorbed twice for periods of half an hour at room temperature with a quarter volume packed and washed sheep erythrocytes.

Human Blood Antiserum. This was prepared by submitting a number of rabbits to courses of intravenous injections of undecomplemented human blood. Most of the rabbits received about 14 injections of about 1 ml. blood over a period of a month. Antisera were decomplemented and, after absorption with sheep cells, these rabbit human blood antisera were stored in 0·5 ml. quantities at —10°C. Haemagglutination titres varying from 1:3,000 to 1:12,000 were obtained. These antisera did not comply with demands laid down by Proom[5] for the precipitin test, as none of them precipitated with human serum diluted more than 1:6,000, while they regularly reacted with high (up to 1:640) dilutions of sera of some unrelated species. One serum (J.N.C.), used in most of the tests described here, had an homologous haemagglutination titre of 1:8,192.

Chimpanzee Blood Antiserum. This was prepared and stored in the same way as the human antiserum described above by substituting chimpanzee blood for human blood.

Due to the limited amount of chimpanzee blood available, only 1 rabbit was used and a haemagglutination titre of 1:2,048 was obtained with homologous antigens.

Sensitization of Erythrocytes. Tanned cells are rather unstable and tend to agglutinate spontaneously. It is found that normal rabbit serum has a stabilizing effect on such cells. The concentration to be used was determined by titration and proved to be 1% NRS in buffered saline. *All* dilutions were accordingly made in this concentration of NRS. Ten ml. of the tannic acid solution was mixed with 10 ml. of a 2·5% suspension of sheep cells and kept at room temperature for 15 minutes. The cells were then washed once with buffered saline and re-suspended in 25 ml. of a 5% solution of the known blood antigen in buffered saline of pH 6·24. Human blood was used in all

instances except in the experiment presented in Fig. 4. After an interval of 15 minutes the cells were again washed once with a 0·4% solution of NRS and finally suspended in 15 ml. of 0·4% NRS in 15 ml. centrifuge tube. The 0·4% rabbit serum protects the sensitized cells against haemolysis during the period of washing.[2] After standing for about an hour any auto-agglutinated cells will usually have sedimented. This deposit was discarded and the supernatant used as an indicator for the test.

The Absorption Test

Triplicate tests were performed in Perspex trays 1 foot × 1 foot × 1 1/16 inch containing 15 rows of 15 round-bottomed holes measuring 1 inch × 3/8 inch. The antigen in one of these tests was an extract of the known blood stain (human blood in all experiments except in the experiment presented in Fig. 4.). In another test the antigen consisted of the unidentified stain

HUMAN BLOOD STAIN EXTRACT	HUMAN BLOOD ANTISERUM												
	2	4	8	16	32	64	128	256	512	1024	2048	4096	1% N.R.S.
2	—	—	—	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—	—	—	—	—	—	—
32	—	—	—	—	—	—	—	—	—	—	—	—	—
64	—	—	—	—	—	—	—	—	—	—	—	—	—
128	—	—	—	—	—	—	—	—	—	—	—	—	—
256	+	—	—	—	—	—	—	—	—	—	—	—	—
512	+	+	+	—	—	—	—	—	—	—	—	—	—
1024	+	+	+	+	—	—	—	—	—	—	—	—	—
2048	+	+	+	+	+	—	—	—	—	—	—	—	—
4096	+	+	+	+	+	+	+	+	+	—	—	—	—
1% N.R.S.	+	+	+	+	+	+	+	+	+	+	+	+	—

Fig. 1. Absorption test of human blood antiserum (J.N.C.) with human blood stain extract.
+ = Agglutination.
— = No agglutination.
Sheep erythrocytes sensitized with human blood used as indicator of annulment.
Figures are reciprocals of dilutions.

extract while in the third test it was a saline extract of unstained linen. 0·2 ml. volumes of geometric dilutions of each of these antigens (arranged in checkerboard fashion in separate trays) were incubated at 37°C. with equal volumes of geometrical dilutions of the known antiserum.

Two series of controls were put up on each tray. The one consisted of various dilutions of the particular antigen in 1% NRS while the other was comprised of the antiserum dilutions. After one hour, 0·05 ml. of erythrocytes sensitized with the known antigen were added to each mixture and to the controls. After thorough shaking the tests were left at room temperature for 2 hours before reading.

The arrangement of the test and controls can best be understood from an inspection of the tables presented. Results are read entirely according to the sedimentation of the red cells on lines advocated for interpreting haemagglutination by influenza viruses.[6] A diffuse even layer of cells covering the

HUMAN BLOOD ANTISERUM

CHIMPANZEE BLOOD STAIN EXTRACT	2	4	8	16	32	64	128	256	512	1024	2048	4096	1% N.R.S.
2	+	+	+	+	+	+	+	+	+	+	+	+	−
4	+	+	+	+	+	+	+	+	+	+	+	+	−
8	+	+	+	+	+	+	+	+	+	+	+	+	−
16	+	+	+	+	+	+	+	+	+	+	+	+	−
32	+	+	+	+	+	+	+	+	+	+	+	+	−
64	+	+	+	+	+	+	+	+	+	+	+	+	−
128	+	+	+	+	+	+	+	+	+	+	+	+	−
256	+	+	+	+	+	+	+	+	+	+	+	+	−
512	+	+	+	+	+	+	+	+	+	+	+	+	−
1024	+	+	+	+	+	+	+	+	+	+	+	+	−
2048	+	+	+	+	+	+	+	+	+	+	+	+	−
4096	+	+	+	+	+	+	+	+	+	+	+	+	−
1% N.R.S.	+	+	+	+	+	+	+	+	+	+	+	+	−

❷

Fig. 2. Absorption test of human blood antiserum (J.N.C.) with chimpanzee blood stain extract.
+ = Agglutination.
− = No agglutination.
Sheep erythrocytes sensitized with human blood used as indicator of annulment.
Figures are reciprocals of dilutions.

hemispherical base of the container is positive agglutination, while any tendency towards ring formation is considered negative.

To determine how the human blood antiserum used in this series of experiments compares with the human anti-globulin serum previously used, samples of the 2 types of sera were adjusted to the same (1:8,192) haemagglutination titre. The erythrocytes employed were coated with human blood. Identical absorption tests of these 2 sera with chimpanzee blood showed the subsequent haemagglutination titre to be invariably higher (1—4 tubes) when human blood antiserum was used.

Figs. 1—5 are representative of results obtained in the differentiation of blood stains. Fig. 1 shows the result of absorbing human blood antiserum with human blood stains. Fig. 2 illustrates the results of absorbing human blood antiserum with chimpanzee blood stains and Fig. 3 represents the

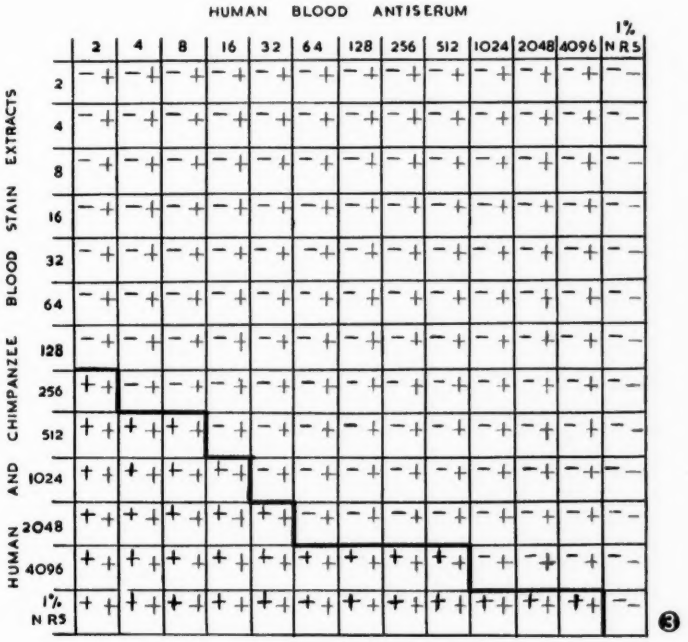

Fig. 3. Superimposed absorption tests of human blood antiserum (J.N.C.) by human and chimpanzee blood stain extracts respectively.
+ = Agglutination in human blood stain test.
— = No agglutination in human blood stain test.
+ = Agglutination in chimpanzee blood stain test.
— = No agglutination in chimpanzee blood stain test.
Sheep erythrocytes sensitized with human blood used as indicator of annulment.
Figures are reciprocals of dilutions.

composite result. Figs. 4 and 5 are of the latter type, with the results of the 2 components of each experiment superimposed to facilitate comparison. Saline extracts of unstained linen never showed any absorption of antisera and are not presented. Fig. 5 is representative of all other tests performed. The various animal stains never gave complete absorption of human blood antisera. The result was that these tests always showed agglutination in all tubes except in the antigen controls. For a contrast, see the upper right corner zone of the experiment presented in Fig. 4, where the dilutions of human blood stains annul the low titre chimpanzee blood antiserum completely. Corresponding human blood stain absorptions of human blood antisera, on the other hand, show patterns very much confined to the lower left corner zone of the checkerboard experiment. Slight irregularities in

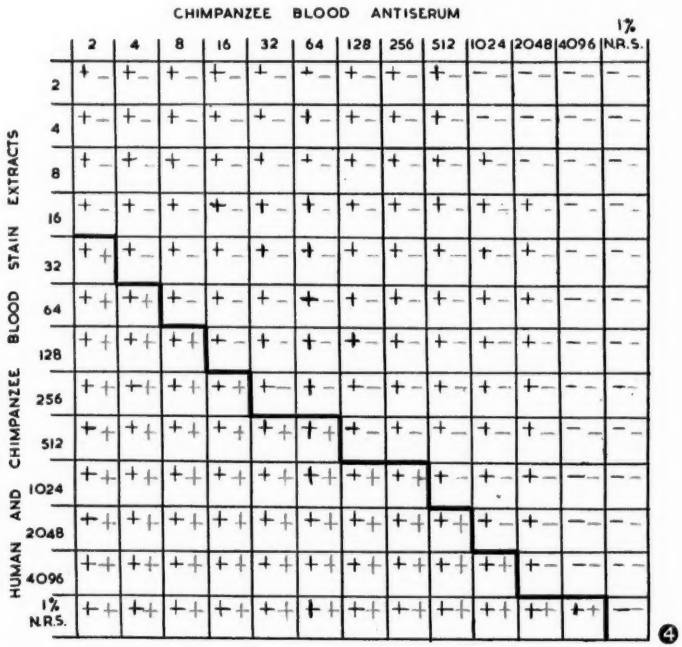

Fig. 4. Superimposed absorption test of chimpanzee blood antiserum with human and chimpanzee blood stain extracts respectively. Haemagglutination titre of antiserum 1:2048.
+ = Agglutination in human blood stain test.
— = No agglutination in human blood stain test.
+ = Agglutination in chimpanzee blood stain test.
– = No agglutination in chimpanzee blood stain test.
Sheep erythrocytes sensitized with chimpanzee blood used as indicator of annulment.
Figures are reciprocals of dilutions.

the step-like outline of the agglutination pattern (indicated by bold lines in the tables) are not constant and can almost certainly be ascribed to pipetting errors. On the basis of the experiments reported here, clear-cut distinctions between human and all animal blood stains can always be made provided the stain is not more than 3 months old.

Discussion

As this test does not attempt to prove identity of antigens but rather to demonstrate non-identity, it is exempted from the obligation of performing the otherwise imperative reciprocal absorption tests. In this restricted field it has been very successful. Clear-cut results were obtained with all blood stains tested including the nice demonstration of differences existing between chimpanzee and human bloods (Figs. 3 and 4).

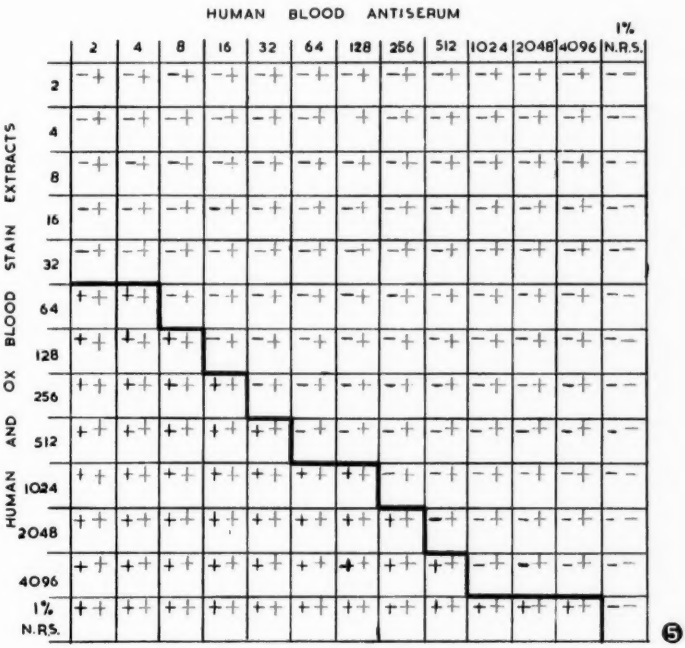

Fig. 5. Superimposed absorption tests of human blood antiserum (J.N.C.) by human and ox blood stain extracts.
+ = Agglutination in human blood stain test.
— = No agglutination in human blood stain test.
+ = Agglutination in ox blood stain test.
— = No agglutination in ox blood stain test.
Sheep erythrocytes sensitized with human blood used as indicator of annulment.
Figures are reciprocals of dilutions.

The replacement of a Coombs' rabbit human antiglobulin serum by one possessing a broader spectrum in the form of human blood antiserum has apparently been justified, although results of actual tests are not strictly comparable with those previously reported.[1]

In an extensive series of experiments, not reported here in detail, the possibility of employing a more suitable red blood cell than that of the sheep was investigated. When using sheep cells, human blood (at least) must be absorbed to remove corresponding agglutinins. This is a disadvantage when dealing with small volumes of bloody extract, some of which is always lost during absorption. However, in practice, there is no way out of this difficulty as even with human O Rh negative cells or Forssman-deficient rabbit cells (which then obviate the need for absorption in cases of human blood stains) the agglutinating potential of an unknown blood extract will not be known, and an absorption will have to be carried out.

Extensive haemolysis or auto-agglutination of the indicator system still occasionally marred experiments and sheep, rabbit, ox and human O Rh negative cells were tested to determine which would best stand the process of tanning while still being a sensitive indicator of unabsorbed antibody. Sheep and human O Rh negative erythrocytes proved the most satisfactory. It was decided to use sheep cells as these were more readily obtainable. By reducing the concentration of tannic acid in the tanning mixture and by altering the pH of the buffered saline slightly, the abovementioned disturbances were reduced to a minimum.

In 1949 Wiener and his associates[7] reported that treatment with high dilutions of human serum annulled the capacity of an anti-human globulin serum to agglutinate human red cells coated with incomplete iso-antibody. This is a property not possessed by the sera of several other species. This test has been subsequently developed by Allison and Morton[8] and Anderson[9],[10], but it has the disadvantage of requiring incomplete antibodies to sensitize erythrocytes, and appears to be less specific than the one described here.

The specificity of the conventional one-tube precipitin test for human blood depends (amongst other things) on the fact that group precipitinogens of unrelated species can usually be diluted out of blood stains and, if any precipitate results from the use of this diluted antigen, it is regarded as being indicative of absolute (or near absolute) correspondence of antigen and antiserum. It is felt that conclusions from the present test may be drawn with greater confidence because this test is interpreted according to the pattern of agglutination.

Reference to Fig. 1 will show that if the human blood stain extract used to absorb human blood antiserum was diluted about 1:16 before use, the agglutination pattern would extend into the upper right corner zone of the checkerboard and so obscure any real distinction between human and

chimpanzee blood (Fig. 2). The only way to avoid misinterpretation of human blood stains in routine applications of the proposed test is to confine the patterns of human blood agglutination to the lower left corner zone by ensuring that:

1. The protein concentration in the unidentified stain extract is of a similar order to that of the protein concentration in the human blood stain extract by dilution with buffered saline if necessary;
2. This concentration of protein be as high as possible.

As the patterns of agglutination of various animal blood extracts extend over the whole range of dilutions, only excessively high concentrations of these extracts may cause their patterns to recede from the upper right corner zone.

The use of a low-titre rabbit antiserum also directs the pattern of haemagglutination of both human and animal blood in the direction of the bottom left corner zone. This is definitely not as critical as adjustment of the protein concentrations of antigens referred to above, as adequate differentiation was obtained (in various experiments not reported here) by using rabbit human blood antisera with haemagglutination titres varying from 1:3,000 to 1:12,000. This is in marked contrast to the exacting demands of the antisera used for identifiying human stains when the precipitin test [11] is used.

A rather striking, and at first somewhat unexpected, feature of all the animal absorption tests is the identity of their agglutination patterns seen over the wide range of dilutions employed. Thus the ox blood stain absorption pattern of human blood antiserum is apparently identical with that of the phylogenetically distant chimpanzee. The answer to this observation is possibly to be found in the exhaustive work of Anderson[9] and also of Landsteiner and Van der Scheer[12], who have demonstrated that an antiserum contains antibodies of different specificities and that, although an antiserum may react in high dilutions with a non-corresponding antigen, such reactions are caused by a relatively small portion of the antibodies since the majority of antibodies are highly specific. While the fraction of non-specific antibodies in human blood antiserum may be sufficient to marr the ordinary precipitin test (or direct haemagglutination test) the specific antibodies present will, in an absorption test, still be sufficient to agglutinate the homologous antigen on red blood cells to high titre irrespective of the phylogenetic position of the non-homologous blood antigens used for absorption.

Summary

Experiments are reported in which various animal blood stains on linen are differentiated from human blood stains on similar material.

The test is a slight modification of a previously reported absorption test in which agglutination of erythrocytes, sensitized with the homologous antigen, is used to indicate failure of antiserum annulment.

Differentiation from the human was adequate with all stain extracts including those of chimpanzee blood.

The shortcomings and the advantages of the test are discussed.

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Pathological Findings Following Unsuccessful Ejection From High Speed Aircraft*

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Experience has shown that a thorough post-mortem examination of the victims of a fatal aircraft accident can often facilitate the reconstruction of the accident. This applies most particularly to the unsuccessful escape during flight. Where an ejection seat has been used, the reconstruction of events is of paramount importance, because this piece of apparatus is comparatively new and it is vital that every particle of evidence relating to its operation should be available to the designers and users. The purpose of this paper, which is in no way intended as a full review of the physiology of seat ejection, is to indicate to the pathologist without aviation experience some specific ways in which his examination can be of value in this respect.

The Standard British Ejection Seat

Put simply, in using the British ejection seat, seat and occupant are fired from the aircraft by means of an explosive charge which results, in the ordinary type of aircraft, in a velocity of 60 feet per second and a peak acceleration of 21 G. Half a second following separation of the seat from the aircraft a bullet is automatically fired which deploys a controller drogue which, in turn, extracts a stabilizing drogue as a result of wind resistance. Pilot and seat then fall to a height of 10,000 feet, at which point the seat straps are released and the stabilizing drogue deploys the pilot's personal parachute, the seat falling away. In the event of the ejection taking place below 10,000 feet, automatic separation of the seat takes place 3 seconds after deployment of the stabilizer. Provision is made for the manual control of parachute deployment in the event of automatic failure. The whole sequence is illustrated diagrammatically in Fig. 1, which also shows a recent refinement in the form of cockpit canopy jettison as an integral part of ejection. This method of canopy jettison is not, as yet, in general use; indeed, ejection through the canopy is perfectly feasible provided that Perspex structures only are involved.

If all goes well, the result should be a smooth ride down with only one conscious effort required from the pilot—that is the actual firing of the

* This paper was originally presented at the International Meeting of Forensic Pathology, Brussels, July 1957.

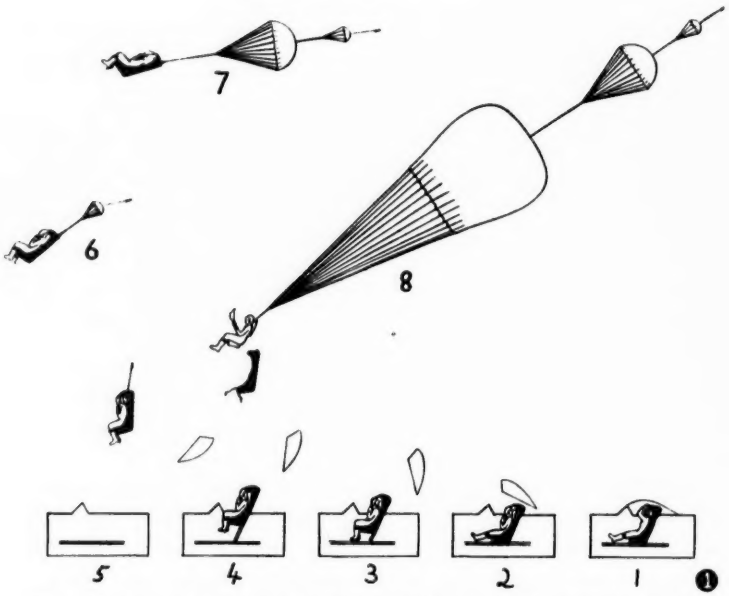
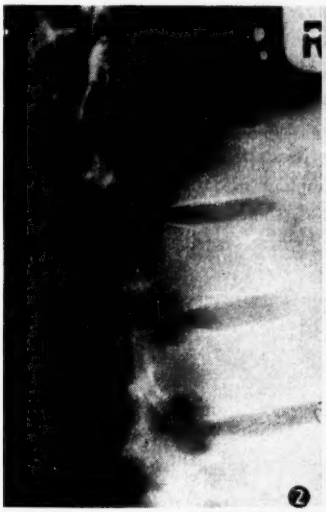


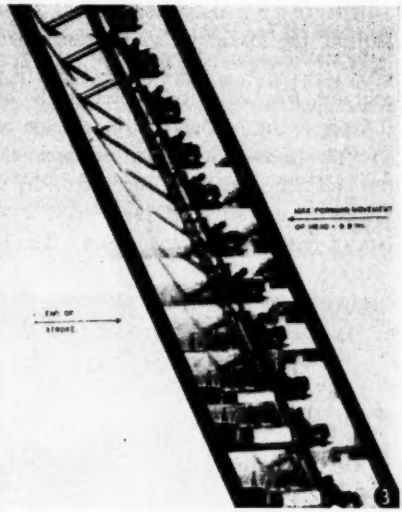
Fig. 1. Diagrammatic representation of a normal ejection. Reading from the bottom right hand corner the stages are (1) Seat firing. (2) The canopy separates. (3) The seat begins to move on its rails and the leg restrainers operate. (4) Cockpit clearance. (5) The drogue gun fires its bullet. (6) Controller drogue extracted by bullet. (7) Stabilizing drogue extracted by controller. (8) Seat separation and deployment of personal parachute. (Reproduced by courtesy of Martin-Baker Aircraft Co.)

seat. Difficulties may, however, occur and the successful analysis of a failed ejection depends both on a meticulous autopsy and on an understanding of the potential pathology from each stage of the ejection sequence. I propose to take each of these in turn.

Potential Sources of Injury

First, the forces applied to the buttocks at the time of seat firing are necessarily considerable and in some ways comparable to a moderately severe crash landing, in which compression fracture of the lumbar spine is a common finding.[1-3] Such fractures were found after using the earlier types of ejection seat. A typical example of this type of fracture is illustrated in Fig. 2. The modern British ejection seat is so designed that, provided seat and man are in apposition at the time of initial thrust, fracture of the lumbar spine will not occur.[4] The most likely reason for failure in this connexion is ejection in the inverted position and a finding of crush fracture of the lumbar spine at autopsy in escape cases must be taken as





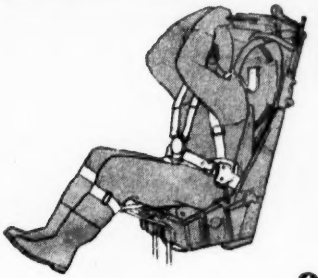


Fig. 2. Radiograph showing crush fracture of the 11th and 12th thoracic vertebrae.

Fig. 3. Cine camera photographs of practice ejection on the test rig. The effect of neck flexion in the absence of head restraint is well shown. (Courtesy of R.A.E., Farnborough).

Fig. 4. Seat firing position. (Courtesy of Martin-Baker Aircraft Co.).

highly suggestive of an abnormal escape attitude. The same result can, of course, arise following an ejection in the normal attitude if a 'springy' seat, of unofficial pattern, is used, and it is also to be expected if the subject is already flexed at the time of firing, as might occur in an inadvertent ejection.[4] Similar considerations apply to bruising of the deep tissues over the ischial tuberosities. I have never been able to find this following a normal ejection; but, on one occasion, severe bilateral bruising was found following inverted ejection and, on 2 occasions, unilateral bruising supported a strong non-medical suspicion of lateral ejection. While, at present, the evidence is too slender to allow one to be dogmatic about the significance of this sign, it should always be looked for in fatal escape attempts.

Due to the position of the centre of gravity of the head, the forces acting at the moment of seat firing will tend to flex the head forcibly on the chest.

This tendency is illustrated in Fig. 3. With the shoulders supported by a harness, the maximum stress from this cause is laid on the spine at the level T 2—T 4.[5] With high rates of acceleration and a lack of head restraint, fracture at this point might be anticipated. In the British seat this possibility is countered by the use of a head blind for seat firing (Fig. 4). This method of seat firing forces the head back against the seat head rest and also gives some support to the shoulder girdle.[6] The post-mortem finding of a fracture at the level T 2—T 4, therefore, strongly suggests a lack of head restraint at ejection. The following case illustrates this point.

Fig. 5. Superficial scratching on the left shoulder of Case 1, believed to be due to scoring by the head blind.

Fig. 6. Minimal chin injuries in Case 1.

Fig. 7. Chin injury of moderate severity believed to be due to acute head flexion on impact with ground.

Fig. 8. A similar injury of more severe degree.

Case 1. Two aircraft collided in mid-air. One lost a wing and tumbled severely. The pilot ejected but his parachute failed to develop completely and he was dead when found on the ground. An eye witness stated that the pilot looked either unconscious or dead as he descended, but nevertheless the case was considered to be one of parachute failure and death from ground impact. At autopsy, there was a fracture of the upper thoracic spine. Examination of the buttocks suggested a lateral ejection and the major fractures had all the qualities of being inflicted after death. In addition, the left shoulder showed some very superficial scratches which could well have been caused by scoring of the blind over the skin in that region (Fig. 5). The post-mortem examination thus indicated the true nature of the fatality, the gyrations of the aircraft causing the pilot to pull the blind over his shoulder instead of over his head.

Severe chin lesions might also be expected in such cases. Fig. 6 shows the chin from the man just described, where the lesions are certainly not gross, despite severe head flexion. The appearances should be contrasted with Figs. 7 and 8, both of which were associated with fractures of the upper cervical spine. The genesis of such gross lesions is therefore different and is believed to be associated with acute flexion of the head on unsupported shoulders on impact with the ground.

The second opportunity for the production of pathological lesions lies in fouling of the cockpit or canopy at ejection. In fact, such lesions are extremely rare. In some circumstances, however, the differentiation of these injuries may be of the greatest importance, particularly when there is evidence of failure of manual controls.

Case 2 illustrates this point. A navigator of a bomber, equipped with a seat requiring manual separation of the occupant, fell from 21,000 feet and was found still in his seat with the parachute open beneath his buttocks. A fracture of his vault was present and it was suggested that this had arisen at the time of the ejection and had prevented the correct use of his manual controls. Post-mortem examination showed, however, that the fracture of the vault arose from internal forces sustained at the time of ground impact. The fatality thus arose from incorrect use of controls due to disorientation during descent.

A third potential for injury production lies in the flailing effects on the limbs following contact with a severe air stream. The commonest form of this type of injury is forcible abduction of the knees and this has been circumvented by the use of automatic leg restraint. Without such restraint, the critical speed of ejection from this aspect lies somewhere between 350 and 450 knots and post-mortem evidence of flailing injuries can give a most useful indication of the speed and attitude of the aircraft when escape was attempted.

Case 3 illustrates this. A fighter pilot ejected in unknown circumstances at night and, in fact, fell to earth without a parachute. At autopsy a series of bruises was found on the inner aspect of the right arm and there was abduction fracture of the necks of the left humerus and femur. It was possible to reconstruct the ejection attitude as shown in Fig. 9. All the post-mortem evidence pointed to a very high speed ejection, possibly associated with rotation.

Fig. 9. Diagrammatic representation of the ejection attitude in Case 3. There is rotation to the left with flailing of the left arm and leg and the right arm has been flung over the head.

It is clear that differentiation of such injuries from those due to terminal trauma is necessary and, since this is so fundamental to the analysis of a failed escape, it will be considered separately.

In the event of the stabilizing drogue failing to develop, tumbling, or rapid rotation about the centre of gravity of the body, may occur. A spin of 140 r.p.m. through the heart will lead to unconsciousness in 3–10 seconds[7, 8] and this will clearly influence the subsequent use of emergency manual controls. The most likely pathological finding of tumbling is subconjunctival haemorrhage, which is said to occur as a result of 90 r.p.m. for 3–10 seconds, but is not absolutely specific for tumbling. Fig. 10 shows haemorrhages believed, but not proved, to be the result of similar forces in a failed escape. Additionally, tumbling due to drogue failure may result in severe injury from impact with the seat following automatic separation and a case has been seen where death was believed to be due to penetration of the heart by broken ribs resulting from this cause (Fig. 11).

If ejection is accomplished at a speed in excess of 350 knots, it is probable that the emergency oxygen supply will be lost and, at high altitude, this could result in unconsciousness or even death. For this reason, in the modern seat, personal parachute development is intentionally delayed until a height of 10,000 feet is reached, the drogue allowing a stabilized descent to this height at a rate of 116 feet per second.[9] The post-mortem diagnosis of death from anoxia in the presence of severe terminal trauma is, in my opinion, extremely difficult. The biochemical test, described by Franks,[10] may have a place but at present can only be regarded as an experimental

procedure. Moreover, in a reasonably large series of aviation deaths, I have never been able to demonstrate the characteristic watery vacuolation of the liver in acute anoxia which has been so widely described.[11, 12]

Parachute opening shock should not produce injury in a normal ejection as the system of drogues is specifically designed to reduce the snatch load. Fig. 12, which is not from an ejection seat escape, shows severe parachute opening trauma to the genitals. Such a finding following ejection would strongly suggest a fault in the sequence.

Finally, in the fatal escape attempt, there will be the pathological findings due to terminal impact with the ground. The practical interest here centres on the possibility of assessing the distance of the fall or alternatively the degree of parachute development achieved during the escape. The main internal findings in some fatal ejections, correlated with the approximate decelerative force applied to the body, are summarized in Table 1. The actual lesions discovered are, of course, to be anticipated and have been described previously in aircraft accidents.[13, 14] There is no absolute correlation between degrees of internal injury and the decelerative force imposed but, while one can seldom be dogmatic, increasing experience has demonstrated how the overall picture of the internal injuries can often give a very fair idea of the conditions surrounding a fall from a height.

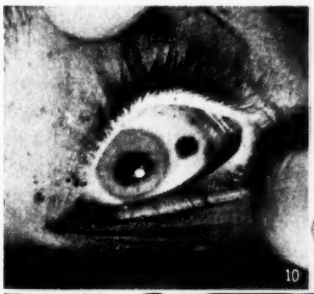


Fig. 10. Subconjunctival haemorrhages probably resulting from 'negative' acceleration (i.e. the direction of force is towards the head).

Fig. 11. Penetration of the heart in three places by broken ribs resulting from seat fouling during descent.

Fig. 12. Laceration of penis and scrotum due to parachute opening shock.

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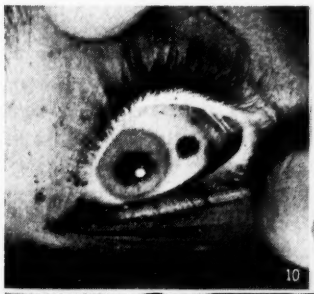

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Table 1: Internal Injuries Associated with Terminal Ground Impact
Fatal Attempted Ejection Escapes

Case Reference	*Estimated Maximum Force ('G')*	*Pulmonary Haemorrhage*	*Haemothorax (Ml.)*		*Laceration of Heart*		*Rupture of*		*Degree of Injury to*				*Grade of Pulmonary Fat Embolism*
			Left	*Right*	*Auricle*	*Ventricle*	*Aorta*	*Diaphragm*	*Liver*	*Spleen*	*Kidney*	*Bowel*	
A	500	++	250	500	+	++	—	—	++	+	++	++	2
B	450	++	10	—	+	—	+	—	++	+	++	++	2
C	210	++	50	50	?	?	—	+	++	++	++	++	2
D	150	++	200	250	±	—	—	—	++	++	+	++	2
E	150	++	200	300	+	—	—	—	+	++	+	—	1
F	100	++	100	25	±	—	—	+	—	—	—	+	3
G	100	++	800	800	+	—	+	—	++	—	++	+	3
H	50	++	—	50	—	±	—	±	±	—	±	+	3
I	50	++	600	500	—	—	+	—	++	+	—	—	1
J	50	++	100	—	—	—	—	—	+	—	—	±	3
K	30	+	10	10	—	—	—	—	—	—	—	—	3
L	5	±	—	—	—	—	—	±	—	—	—	—	4

Timing of Injuries

Having considered in some detail the various ways in which pathological findings can be produced during the ejection sequence, it is apparent that the analysis of the failed escape depends largely on the timing of the injuries sustained.

Table 2: Type of Accident and Degree of Pulmonary Fat Embolism

Grade of Pulmonary Fat Embolism	*Number of Cases*				
	Impact	*Voluntary Ejection*	*Involuntary Ejection*	*Failed Unassisted Escape*	*Others*
0	12	—	—	2	1
1	4	2	2	1	—
2	3	3	2	—	1
3	2	2	—	1	—
4	—	1	—	—	—

Armstrong, Fryer, Stewart and Whittingham[15] described pulmonary fat embolism in 12 out of 19 bodies from the Comet disasters and assumed this to be evidence of a finite time between injury and death. In Table 2, which does not include all cases to date, I have assessed the degrees of pulmonary fat embolism found following various types of aircraft accident. It is seen that some degree of fat embolism appears in all cases following the use of an ejection seat. This cannot mean that severe injury prior to terminal impact has occurred in all such cases, though it may be possible that the jar given to the lumbar spine at the time of the initial thrust is sufficient to cause minor degrees of fat embolism.[16] On the other hand, I have often demonstrated fat embolism in cases believed, perhaps erroneously, to have been examples of instantaneous death due to multiple severe injury. Moreover, Table 1 shows that the assessment of pulmonary findings is further complicated by the apparent correlation between the more severe degrees of embolism and the milder forces, which could be interpreted as indicating increased survival time. The presence and degree of pulmonary fat embolism is, therefore, influenced by so many variables that, in our present state of knowledge, it is difficult to utilize its finding as a means for timing injuries.

It appears that the diagnosis of ante-mortem or post-mortem injury must rest largely on the local reaction. The difficulties surrounding this observation have recently been described by Robertson and Mansfield[17] and, in the type of case under discussion, the difficulties are increased by the minimum time available for changes to occur. So many variables arise in the macroscopic evaluation of degrees of vital reaction that it seems that abso-

Fig. 13. Ante-mortem and post-mortem fractures opened at autopsy. The fracture of the humerus is quite bloodless while that of the radius and ulna is deeply haemorrhagic.

lute significance can, in the absence of highly specialized techniques, only be given to a complete absence of haemorrhage associated with a severe fracture. Fig. 13 shows 2 fractures, opened at autopsy, in the arm of an ejection seat fatality. That associated with the elbow joint is deeply haemorrhagic while that of the humerus is wholly bloodless. It was inferred that the former was caused by seat fouling during descent while the latter occurred *post mortem* at ground impact. In the circumstances of this particular accident there was no contra-indication to such an analysis. Nevertheless, even this limited view of the significance of the presence or absence of local reaction to injury is open to criticism and a well proved method for the timing of injuries remains one of the most important requirements of the traumatic pathologist.

Conclusion

The purpose of this paper has been solely to demonstrate the value of the autopsy examination in the analysis of ejection escape fatalities. In Table 3, the injury or death-producing potential is briefly recapitulated. The evaluation of the injuries sustained requires considerable time and patience but, because such deaths are so uncommon, a meticulous post-mortem examination should invariably be performed. It is submitted that, in few other instances, can the isolated post-mortem examination provide more surely the means of saving human lives in the future.

Table 3: Potential Causes of Injury or Death following Unsuccessful Ejection

Cause	*Possible Injuries*
1. Seat firing	Fracture lumbar or thoracic spine.
2. Aircraft fouling	Fractured skull.
	Injuries to limbs.
3. Air Stream	Injuries to limbs.
4. Tumbling	Subconjunctival haemorrhages.
	Trauma due to seat or rigging fouling.
5. Anoxia	General.
6. Parachute opening shock	Laceration of groin.
7. Terminal impact	Generalized external and internal.

Summary

The operation of the standard British ejection seat is outlined.

A fatality arising from an attempted escape by ejection indicates a failure at some point in the sequence. The potential pathological findings are correlated with the various stages in the ejection and illustrations are given showing how the post-mortem examination may disclose the circumstances surrounding the unsuccessful escape.

General observations are made on the timing of injuries on the basis of the degree of pulmonary fat embolism and of local reaction to injury.

The importance of the post-mortem examination in these rare causes of death is stressed as an aspect of preventive medicine.

In the majority of instances, I have attended the autopsies as a privileged observer only. I am greatly indebted to the following pathologists who have allowed me to quote or use illustrations from cases under their jurisdiction: Dr. Morgan Hall, *Norwich*; Dr. Gerald Evans, *Bangor*; Dr. J. W. Lacey, *Chelmsford;* and Dr. A. B. Lintott, *Ipswich.* My thanks are also due to numerous officers of the R.A.F. Institute of Aviation Medicine for help and advice; to Group Captain Wynroe for the provision of Fig. 2; and to the Director General of Medical Services, Royal Air Force, who has given permission to submit this paper for publication.

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Blood Sugar Levels in Starvation

With and Without Alcoholic Drinks

Alfred J. Coulthard
London, England

The importance of a regular and ample supply of sugar to the brain, by means of the blood stream, is in no danger of being ignored when a biochemical explanation is sought for anti-social behaviour. Conflicting views are held regarding the blood sugar level in starvation, a frequent assumption being that it must fall progressively lower as time passes without food. The effects of abstinence from food upon the blood sugar level are often grossly over-estimated, normal persons deprived of food being able to maintain a relatively constant blood sugar level for a considerable time after the initial fall; certainly for many days, and probably very much longer.

In clinical circles, of course, the picture for minor fasting (Fig. 1B) (such as going without breakfast) is extremely familiar, if only because of the blood sugar curve, a common test for diabetes mellitus. Little work appears to have been done until comparatively recently, however, upon the effects of starvation or for periods of fasting much longer than the above.

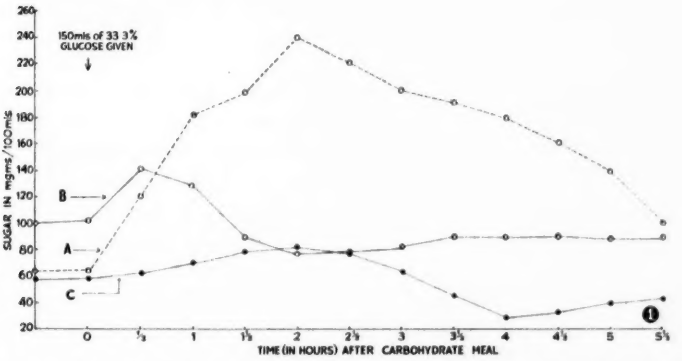

Fig. 1. Blood sugar in various fasting conditions broken by carbohydrate meal.

A: After 7 days' starvation. (Partly based on Woolenberger and Linton.[20] Coulthard's modification of King's original method).

B. Normal well-fed subject (Coulthard. By King's original micromethod —modified).

C. In semi-starvation of famine victims (Fliederbaum's subject[14] by the method of Hagedorn and Jensen).

Blood Sugar Levels in Chronic Semi-Starvation

This is not an easy condition to simulate or to investigate at first hand. World War II, however, provided a good deal of evidence, some well documented. Thus citizens and prisoners at Wuppertal in Sieberg in 1946, all of whom were under-nourished and had lost weight up to 55 kilogrammes gave a normal fasting blood sugar, a normal oral glucose tolerance and normal adrenaline and insulin sensitivity (tolerance) tests.[1]

The Blood Sugar Level in Longer-Period Fasting

The results of a number of investigations into these 'longer periods' varying from 18–36 hours of starvation, show findings similar to the typical one illustrated in Fig. 2, and these, along with other investigations, suggest that in the normal subject the blood sugar level may indeed fall during the first few days, reaching a variable level which may be as low as half (more usually two thirds) the normal value.

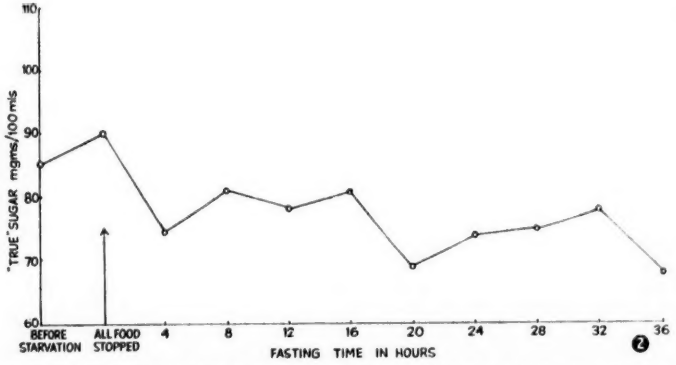

Fig. 2. Blood sugar during longer-period fasting. King's original micro-method (modified).

After the initial period the level is maintained or even rises with more pronounced fasting, presumably because metabolism becomes adapted to fasting conditions.

It should be emphasized that these findings probably represent the normal response, and it is not disputed that occasionally other types of curves[2] may be encountered, even in apparently perfect health.

The effects of starvation upon the blood sugar level have, until recently, not been so widely known, and the absence of regular meals or the existence of a lengthy period since taking food, appeared a reasonably plausible line of defence on the grounds of a resultant hypoglycaemic syndrome; but even in longer periods of fasting it seems very doubtful if symptoms of hypoglycaemia are likely to occur in normal persons without liver damage.

Partly, no doubt, because of the recovery the blood seems able to make after the initial sugar decrease and partly, no doubt, because of the very gradual nature of any further decrease that may take place, such symptoms do not seem to occur even with blood sugar levels of 60 mg. per 100 ml. or even less.

More widespread knowledge about the normal response, however, brought further complications. What was the position if alcoholic drink was consumed during the fasting period? Was the blood sugar lowered as a result or was it, in fact, built up from the alcohol? Both schools of thought existed, and so further investigation was decided upon.

Blood Sugar Levels in Longer-Period Fasts Broken By Alcoholic Drinks

The possibility that ethanol is converted into carbohydrate is a matter of dispute. Forbes and Duncan[3] state positively that this does not happen, whilst Kleiner[4] says:

'It has been thought that it cannot be converted into glycogen but since CO_2 can be utilized in the formation of glycogen, there is no reason to assume that the CO_2 resulting from the breakdown of alcohol cannot be.'

The fate of ethanol in the body is presumably as shown in Fig. 3.

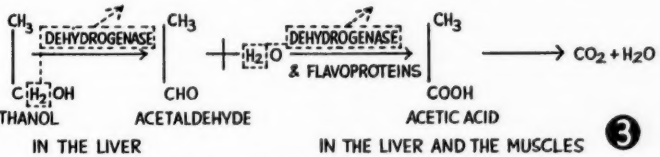

Fig. 3. Presumed fate of ethanol in the body.

However, whatever the destination of the CO_2 in the sphere we are considering, the most important point is to prove or disprove the suggestion that, in the normal person, the blood sugar level is lowered by the intake of ethanol during starvation, and steps were accordingly taken to test this point.

It is of course obvious that the type of drink taken is of great importance, many beverages containing very appreciable amounts of sugar (Table 1). The effects of these could be conjectured fairly accurately and in fact, they behaved much as one would anticipate from the sugar content alone (Fig 4).

The main interest, however, was in ethanol itself, and Fig. 5 shows the results of 4 investigations where 125 ml. of 20% ethanol were given after 14 or more hours of fasting. The blood sugar level was determined at 30-minute intervals for 4 hours.

(As a matter of interest, the blood alcohol levels were also examined simultaneously, but these findings are not relevant to this paper although it may be remarked that these showed the extremely rapid peaking (30

Table 1: Ethanol and Carbohydrate Content of Some Alcoholic Beverages

	Ethanol By Volume %	*Sugar or Maltose*	*Protein*	*Fat*	*Carbohydrate*
			(*Gramme per* 100 *ml.*)		
Malt Beverages					
Ale, Strong	5—9		0·5	Trace	4·8
Beer, Mild	2—5		0·5	Trace	3·0
Beer, Bitter			0·2	Trace	1·7
Cider	5—9			0	1·6
Lager		0·88		0	
Stout	5—9		0·3	Trace	3·9
Wines		*Reducing Sugar*			
Sparkling	11—15	0·02—5·5	0·7— ·07	0	
Dry, White	10—14	0·05—0·3	0·06—0·3	0	0·4
Dry, Red	10—15	0·04—0·2	0·07—0·3	0	0·1
Sweet White	11—21	1·7—17·0	0·02—0·2	0	
Sweet Red ..	13—22	3·0—16·0	0·1 —0·3	0	
Spirits					
Brandy	45—57	0	0	0	Trace
Gin ..	51—59	0	0	0	Trace
Rum	51—59	0	0	0	Trace
Whisky	51—59	0	0	0	Trace

minutes) and relatively high level of blood alcohol associated with the intake of ethanol upon an empty stomach).

Table 2 compares the above with the findings of other workers, and the combined evidence supports the contention that ethanol does not lower the blood sugar if taken during a longer-period fast by a normal person.*

There is indeed some evidence that ethanol may slightly raise the fallen blood sugar in longer-period fasts, and other evidence suggests that it may prevent further falls in blood sugar levels where these are due to normal (or abnormal) metabolic activity. Evidence from other workers as to whether the actual rise might be greater when taking food or when fasting seems contradictory.

Comparisons between Carbohydrate and Ethanol in Conditions of Fasting

In this connection one other finding in extended fasting may be noted. Tennant[13] found that ethanol (in rats) shows a much greater rise in blood sugar after carbohydrate (liver full) than after longer-period fasting (liver

* Although under certain conditions workers in other countries claim to have observed a fall in blood sugar levels following the intake of ethyl alcohol, there appears to be no convincing evidence to shake the above. Thus Bottura *et al.*[11] appear to deal with chronic alcoholics with fatty infiltration of liver, kidneys and heart (on necropsy) and Neves *et al.*[12] were dealing in the main with alcoholics. Out of extensive sifting of the world literature published during the past 25 years, these are the only examples I regard at all seriously. Both are from South America. Earlier still I know of 3 such claims. Two appear in Table 2, but none is really convincing for the reasons given above or for other reasons.

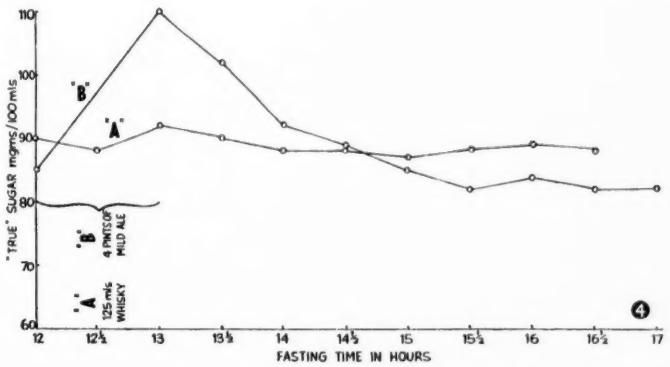

Fig. 4. Blood sugar during fasting 'broken' by alcoholic drinks. Capillary blood by King's original micro-method (modified).

depleted). The intake of a measured amount of carbohydrate after a lengthy fast (liver depleted), however, produces a much greater increase in blood sugar than it would do after a brief fast (liver full, Fig. 6).

It should be noted that this latter observation refers to a normally well-fed subject who is subjected to a longer period of starvation. In the case of chronic semi-starvation (e.g. Nazi concentration camp victims) the reverse appears to be the case, and the consequent rise in level much less than normal (Fliederbaum *et al.*),[14, 16] due no doubt to temporary (or other) impairment of some kind (Fig. 1).

Table 2: Effects of Varying Amounts of Ethanol Upon the Human Blood Sugar Level (Various Workers)

Investigator	*Amount of Ethanol Given*	*Amount of Change (mg. per 100 ml.)*	*Condition of Subject*	*Method of Administration*
Himwich *et al.*[5]	1·5 g./Kg.	+ 10	Fasted	Stomach
Labbe, Nepveux and Chevki[6]	1·0 g./Kg.	+ 12·39	Fasted	Stomach
Hetenyi[7]	16·4 g./Kg.	*Average* 22	Fasted	Stomach
Cavrila and Sparchez[8]	0·4 g./Kg.	—	Fasted	Stomach
Kolta[9]	7·99 g.	*Maximum* 11	Fasted	Stomach
Tennant[10]	0·6 g./Kg. 0·6 g./Kg.	Nil Nil	Fasted Fasted and chocolate	Stomach Stomach
Coulthard	0·43 g./Kg.	Nil or a very slight rise	Fasted	Stomach

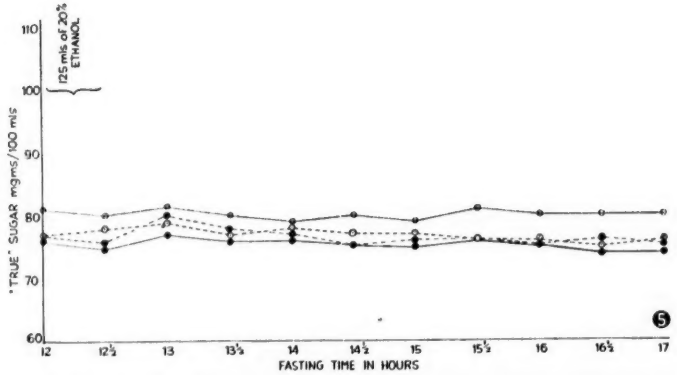

Fig. 5. Blood sugar during fasting 'broken' by ethanol (4 subjects). Capillary blood; method of Asatoor and King.[18]

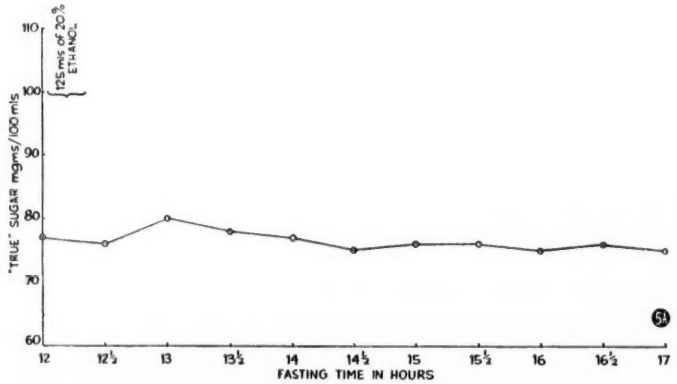

Fig. 5*a*. Blood sugar during fasting 'broken' by ethanol (1 subject). Venous blood: method of Asatoor and King.[18]

In short, whether ethanol is converted into glycogen or not, lowering of the blood sugar seemed an unlikely consequence of its administration (although, of course, in the case of a chronic alcoholic where carbohydrate has been largely replaced by alcohol, the liver glycogen content may well be lowered). As already stated, in some cases the intake of ethanol resulted in a slight rise in the blood sugar level (Figs. 5 and 5A). This occurred from relatively small quantities of ethanol administered when fasting has presumably drawn upon the liver's supply of glycogen.

To sum up: During longer-period fasts (over 12 hours) ethanol may raise the blood sugar somewhat, whilst carbohydrate will definitely increase the blood sugar more than it will in short-period (less than 12 hours) fasts.

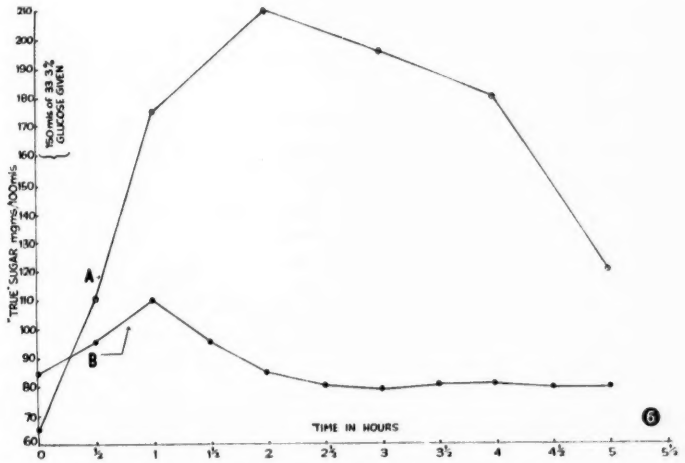

Fig. 6. Effects of carbohydrate upon fasting and non-fasting blood sugar levels, by King's original micro-method (modified).
A: Subject deprived of food for 6 days.
B: Normal well-fed subject.

Tennant[10] believes that where the liver is replete with glycogen, larger amounts of ethanol would bring about large increases, but this is not borne out in his work on humans (Fig. 7)* which is, in the main, in agreement with Jacobsen[15] who considers that ethanol generally speaking, appears not to affect the blood sugar level when this is normal or raised (but may act physiologically as a carbohydrate and prevent hypoglycaemic symptoms from developing when levels have decreased).

To sum up: In short-period fasts (under 12 hours) ethanol seems extremely unlikely to create any sugar decrease and may promote an increase, whilst carbohydrate in short-period fasting will show less increase than in longer period fasting.

A possible explanation for these seemingly contradictory findings (i.e. that carbohydrate taken at later stages of fasting with a presumably depleted liver glycogen store, results in a bigger blood sugar rise than in earlier stages with presumably a fully charged liver) would seem that once the blood commences to rely entirely upon liver glycogen to replenish and maintain its sugar level, it at first fails to deal normally with any belated carbohydrate that reaches the stomach. Ethanol, on the other hand, if it promotes a greater sugar increase in non-fasting as opposed to longer-

* In a personal communication, Tennant[10] thinks the explanation of this apparent discrepancy lies in the practical difficulty of giving humans an amount of ethanol comparable to that producing this effect in rats.

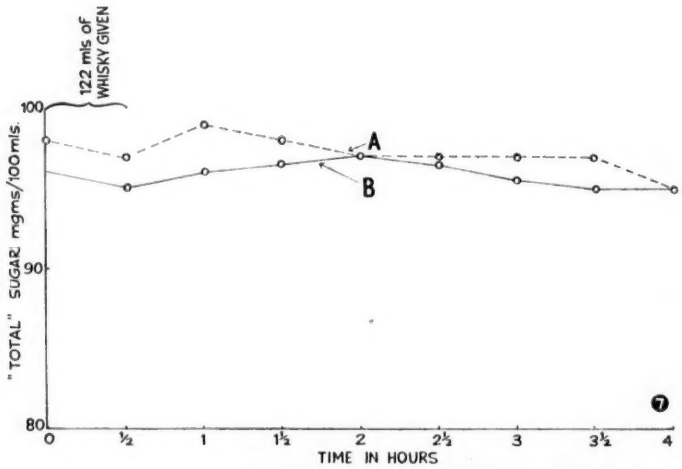

Fig. 7. Effects of ethanol upon fasting and non-fasting blood sugar levels (from Tennant). Capillary blood: Folin's colorimetric method.

A: On an empty stomach.

B: 4 hours after 250 g. of candy.

fasting conditions, does so because of some glycogenolytic action it possesses.

Effects of Methanol upon the Fasting Blood Sugar

There is ample evidence that in the case of methanol drinking, hypoglycaemia does frequently occur, possibly because the toxic products formaldehyde and formic acid (instead of aldehyde and acetic acid) temporarily inhibit glycogenneogenesis and so the replenishment of carbohydrate fails (Fig. 8).

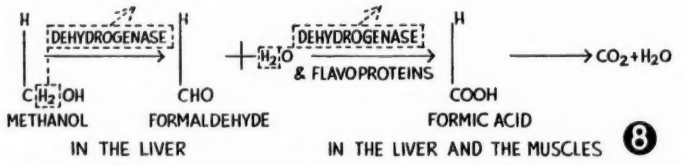

Fig. 8. Presumed fate of methanol in the body.

Although one is not perhaps likely to be confronted with the complication of methanol drinking in the circumstances under discussion, methanol has been mentioned here because some literature dealing with 'alcoholic hypoglycaemias'[17, 18] deals, in fact, with mixtures of ethanol *containing methanol*. The latter almost certainly explains any hypoglycaemia.

Summary

The blood sugar level is apparently not reduced by greatly prolonged restriction of diet.

During a 'longer-period' total fast by a normally well-fed subject with no liver damage, the blood sugar level may show an initial drop, but will then remain relatively constant for many days without further fall and without symptoms of hypoglycaemia, other than hunger. The intake of a single carbohydrate meal during this time will raise the blood sugar much more than would the corresponding amount during a short fast.

The intake of ethanol during this time will almost certainly not lower the blood sugar level, although it may raise it.

There is disagreement about the possibility that ethanol acts as carbohydrate.

Although, at first sight, the effects of ethanol and of carbohydrate appear similar, this is not invariably so and there seems no doubt that the mechanisms by which they may increase blood sugar levels are quite different.

There seems evidence, in fact, that ethanol stimulates glycogenolysis, at any rate when the liver is replete with glycogen.

Contradictory results by different investigators may be due to:

(1) The use of widely varying amounts of ethanol; and

(2) The administration of this at widely varying levels of liver glycogen.

Methods

Blood alcohol determinations were performed by the di-chromate method.

Blood sugar determinations were done by the method of Asatoor and King[19] or by the author's modification of King's original micro-method.[2]

Thanks are due to D. A. Waugh, S. Rogerson and B. Michaelson for observing many unorthodox Fast Days, sometimes assuaged with draughts of ethyl alcohol but more often not.

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Assessment of Age by the Measurement of the Haversian Canals of Human Bones

A Critical Study of the Balthazard and Lebrun Method

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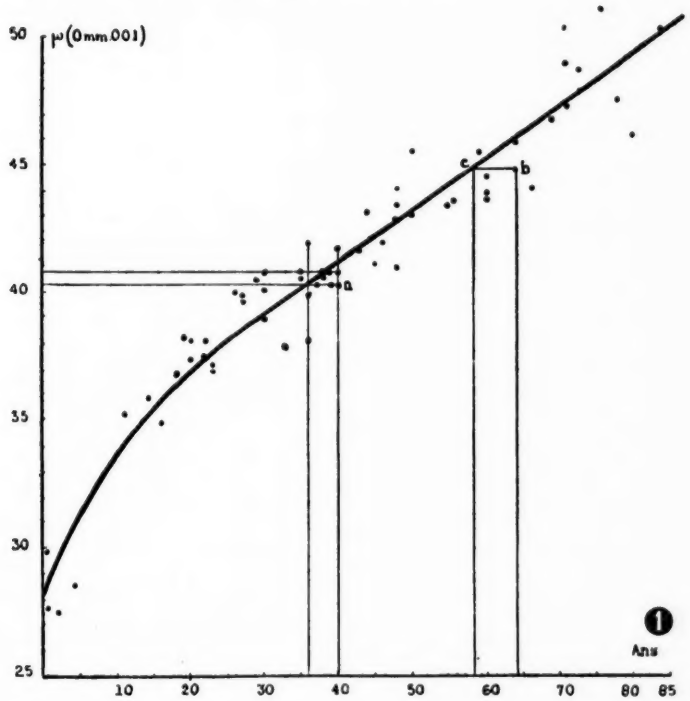
From the Laboratory of Legal Medicine of the University of Ghent, Belgium†

In 1911 Balthazard and Lebrun[1] contended that it was possible to assess the approximate age of a person older than 10 years by the determination of the mean diameter of the Haversian canals (be it of but a single bone or diaphyseal fragment) and described a precise method for doing so. Their



* Senior Technical Assistant.
† Director: Prof. F. Thomas.

carefully worked out technique comprises the following consecutive operations:

When possible, preference is given to the middle of the diaphysis of the tibia.

By means of a fine saw a transverse slice about 1 mm. thick is cut out of the bone. This slice is further thinned out by polishing between 2 fine emery stones.

The section is placed for 3 hours in an aqueous solution of methylene blue.

Table 1

Autopsy Reference	*Real Age in Years*	*Mean Diameter (μ) (Deslypere)*	*Age According to Balthazard (In Years)*	*Mean Diameter (μ) (Baert)*	*Age According to Balthazard (In Years)*
1956/81	4	**44·2**	**54**	**39·2**	**30**
1956/63	17	39·2	30	44·2	54
		38·6	26	39·7	31
1956/80	19	53·9	85	57·6	85
		37·5	**22**	**43·1**	**49**
1956/181	20	44·2	54	48·7	74
		52	**80**	**44·8**	**58**
1956/1	23	39·7	32	40·3	36
		38	24	36	19
1956/59	29	40·3	36	38	24
1956/34	37	55·4	85	49·8	79
		34·1	10	34·1	10
1956/10	43	42·5	44	42	42
1956/131	43	46·4	65	39·7	31
1956/180	43	44·8	58	46·4	65
		41·4	**41**	**48·1**	**72**
1956/55	45	38	24	37·5	22
		42	42	38·6	26
1956/107	45	40·3	36	42·5	45
1955/210	47	42·7	48	42·6	47
1956/28	55	38·6	26	37·5	21
1956/9	57	42·5	45	42·5	45
1956/83	59	40·8	38	44·2	54
		47	**67**	**47·6**	**70**
1956/41	60	53·7	85	43·1	49
1956/49	61	44·8	58	39·7	31
1956/50	64	47·6	70	40·3	36
1956/31	67	38·6	25	37·5	22
1955/218	68	48·1	72	48·7	74
		43·6	52	41·4	41
1956/86	72	45·9	64	41·4	41
1956/54	73	40·3	36	35·2	14
		39·7	31	36·9	19
1956/42	80	46·4	65	40·3	36
			Addendum		
1956/164	The tibia of a full-term foetus provided the following figures:	25·2	0	25·2	0
1956/82	A 9-month-old baby gave the following figures:	**17·3**	**0**	**26·8**	**0**

Then it is washed, rid of the emery particles by brushing, dehydrated in pure alcohol, cleared up in xylol and mounted in Canada balsam between slide and cover glass.

Measurements are made by means of an ocular micrometer.

The Haversian canals are sometimes cut transversely. Sometimes, however, they are sliced obliquely, in which case they will be oval instead of round. Here Balthazard and Lebrun advise that the smaller diameter be measured for accuracy so as to have an exact measurement. One hundred to two hundred canals must be measured, keeping always to the same region of the slide, i.e. in the zone extending between the crista tibialis and the medullary canal. As a rule the diameters increase from the outside to the inside. It is therefore important to measure all the canals from the surface to the medullary canal. The French authors have grouped all their results in a curve (Fig. 1). They concluded that the mean diameter of the Haversian canals increases in direct function with the age.

We have considered it advisable to verify the degree of trustworthiness of this method, which is still honoured in French-speaking countries. We have therefore asked the staff of the Department to provide us with necessary specimens taken from the middle of the tibial diaphysis. Each one was identified by a code number and we were left in complete ignorance of the real age of the deceased. This information was only provided when our own estimations had been completed. The following is a survey of our material (Table 1) which consisted of:

(*a*) A fragment of the tibia from 20 bodies;

(*b*) A fragment of the right and of the left tibia from 6 other bodies, each with a different code number.

Technique

In 24 cases we strictly conformed to Balthazard and Lebrun's instructions as set out. Since their method is extremely long and tedious, we decided to shorten the procedure by resorting to preliminary electrolytic decalcification of the bone. We used both techniques on the same bone in 5 of 24 cases; in 2 other cases only the second one was used. The data relevant to these 7 cases are grouped in Table 2.

The technique is as follows. As previously mentioned, a thin slice of bone is cut out by means of a fine saw and weighed. A platinum wire is bound around it. It is then plunged in a watery solution of 8% formaldehyde acid and 10% HCl, the temperature being maintained at 36° to 40° C. The platinum wire is connected with the positive pole of a 6-volt battery. A second wire is placed separately into the solution, and fixed to the negative pole. This closes the circuit.

After passing the electric current through the slice of bone for 6 hours, the bone is removed, washed, dried and weighed, and the degree of decalcification is assessed.

The fragment is next stuck on a cube of lead and softened in Mollifex. Two or 3 sections of about 20 microns are cut by means of a sliding microtome. The further procedure is as before.

Table 2

Autopsy Reference	*Real Age (In Years)*	*Mean Diameter (μ) (Deslypere)*	*Age According to Balthazard (In Years)*	*Mean Diameter (μ) (Baert)*	*Age According to Balthazard (In Years)*
1956/81	4	**44·2**	**54**	**39·2**	**30**
1956/80	19	53·9	± 85	57·6	± 85
		37·5	**22**	**43·1**	**49**
1956/181	20	44·2	54	48·7	74
		52	**80**	**44·8**	**58**
1956/180	43	44·8	58	46·4	65
		41·4	**41**	**48·1**	**72**
1956/83	59	40·8	38	44·2	54
		47	67	47·6	70
			Addendum		
1956/164	The tibia of a full-term foetus provided the following figures:	25·2	0	25·2	0
1956/82	A 9-month-old baby gave the following figures:	**17·3**	**0**	**26·8**	**0**

Table 3

Autopsy Reference	*Real Age (In Years)*	*Mean Diameter (μ) (Deslypere)*	*Age According to Balthazard (In Years)*	*Mean Diameter (μ) (Baert)*	*Age According to Balthazard (In Years)*
1956/63	17	39·2	30	44·2	54
		38·6	26	39·7	31
1956/1	23	39·7	32	40·3	36
1956/1*		38	24	36	19
1956/34	37	55·4	± 85	49·8	79
		34·1	10	34·1	10
1956/55	45	38	24	37·5	22
		42	42	38·6	26
1955/218*	68	48·1	72	48·7	74
1955/218		43·6	52	41·4	41
1956/54	73	40·3	36	35·2	14
		39·7	31	36·9	19

The instructions of Balthazard and Lebrun were closely followed. At least 100 diameters were measured, starting from the crista tibialis and working toward the medullary canal.

The results of the investigations have been grouped in Tables 1, 2 and 3, and are self-explanatory. As a glance will show, both of us, working independently (in 4 cases), have obtained nearly identical results which were correct in regard to age (1955/210, 1956/10, 1956/82, 1956/164). However, in 16 other cases, the results were unsatisfactory. In 13 the

divergence between the age assessed and the real age was inadmissibly outspoken. In 3, the readings of one of us gave a good approximation, while those of the other gave a poor one. Finally, in 6 cases (Table 3) in which each investigator examined separately a slide of the left tibia and a slide of the right tibia, without knowing they came from the same person, the results were, with the exception of 2 readings (1956/1* and 1955/218*), irreconcilable.

Discussion

From the present investigation it is clear that the method of Balthazard and Lebrun does not justify the hopes these authors placed in it. This is not surprising, since the reasons for the failure had been foreseen by the authors themselves. Quoting Gebhart,[2] they expressly underlined, in their introduction, the possible inequalities in shape and size of the Haversian canals in Man. These failures can be better understood to-day, in the light of the publications of Amprino,[3] Rutishauser,[4] Lacroix[5] and Vincent.[6] These authors have convincingly proved that the Haversian canals not only show considerable individual variations, but that they are subject to continued re-modelling during life. The latter process is the consequence of various pathological influences such as bone trauma and various visceral diseases and connected, moreover, with the organism's changing needs of calcium and phosphorus.

It is to the credit of Balthazard and Lebrun that they undertook their research nearly half a century ago. They did so at a time when everyone considered bone structure as an essentially static concept. In the light of our experience, their method is too unreliable to be of use in routine forensic medicine.

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Toxicologic Study of Chemically Altered Hemoglobins

And Forensic Study of Blood Stains by Paper Electrophoresis*

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With the Technical Assistance of

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and

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Knowledge of normal adult (A), fetal (F), and various abnormal (C, D, E, H, I, J, L, M, S) hemoglobins has been greatly advanced through the use of paper electrophoresis paired with studies of lability to alkali denaturation. Though these methods have opened new avenues of research, knowledge is still incomplete.

These studies were stimulated by two unrelated problems with which we were confronted.

Part I

The first problem revolved around a married couple with clinically evident cyanosis of obscure origin. Laboratory studies included repeated paper electrophoresis of hemoglobin extracted from the patients' venous blood drawn into tubes with oxalate. In each instance, a band was seen on the paper strip which trailed that of normal adult oxyhemoglobin A of control samples. Electrophoresis of artificially prepared nitrous dioxide hemoglobin yielded a band in proximity to that of the patients' blood (Fig. 1). Methemoglobin could not be identified spectroscopically in the blood of the patients. Abundant coliform organisms were identified in stool cultures. The observation led to study of artificially altered hemoglobin.

Materials and Methods

Oxalated blood from healthy blood donors was used as the source of hemoglobin A. The same blood was used for preparation of altered hemoglobins:

Cyanhemoglobin. Hydrogen cyanide, produced by treating powdered sodium cyanide with N 4 hydrochloric acid, was bubbled through 5 ml. blood samples for 15 minutes.

* This investigation was supported by research grant H-2534(C) of the National Heart Institute, N.I.H., U.S.P.H.S., Department of Health, Education, and Welfare.

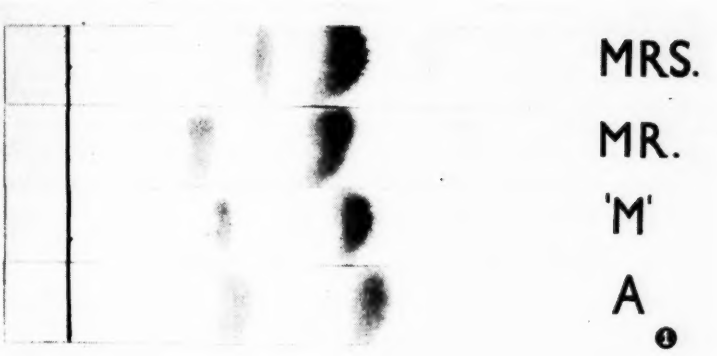

Fig. 1. Elphoregrams of hemoglobin extracted from venous blood of two patients, 'Mrs.' and 'Mr.', of artificial nitrous dioxide hemoglobin 'M', and of normal adult hemoglobin 'A'.

Mobility of main hemoglobin of 'Mrs.', 59; of 'Mr.', 57; of artificial hemoglobin 'M', 61; and of normal hemoglobin 'A', 66 mm. from starting line.

Nitrous Dioxide Hemoglobin. Nitrous oxide from a gas tank used for anesthesia was bubbled through 5 ml. of blood for 5 minutes.

Carbon Monoxide Hemoglobin. Two methods were employed. Either illuminating gas or carbon monoxide, manufactured by treating lumps of potassium ferrocyanide with N 4 hydrochloric acid, were bubbled for 15 minutes through 5 ml. blood samples.

Carbon Dioxide Hemoglobin. Carbon dioxide, obtained by treating powdered calcium carbonate with N 4 hydrochloric acid, was bubbled through 5 ml. blood samples for 15 minutes.

Sulfmethemoglobin. Hydrogen sulfide, produced by treating lumps of iron sulfide with N 4 hydrochloric acid, was bubbled through 5 ml. blood samples for 5 minutes.

Methemoglobin. Fifty mg. of sodium nitrite crystals were added to 2 ml. of oxalated blood for a period of 5 minutes.

Aliquots of blood samples with maximum ('100%') saturation were mixed with the original normal blood to prepare '75%, 50%, 25%, 15%, and 5% saturated' samples. All specimens, i.e. the 6 different concentrates of treated blood and the untreated control blood, were processed by the method of Singer, Chernoff, and Singer[2] for paper electrophoresis.

Electrophoresis took place in a sodium barbital-sodium acetate buffer of pH. 8.6 and ionic strength of 0.1 mho, at 3.2 milliamperes and 110 volts, and at 7–10°C. for 14 hours.

Results

Cyanhemoglobin. In all experiments, the mobility of cyanhemoglobin was slower than that of hemoglobin A. The difference was evident at '25% saturation.' Variations from one preparation to another were negligible (Table 1).

Nitrous Oxide Hemoglobin. Migration rate of all preparations was less than that of normal controls. Trailing was observed at '50% saturation' (Table 1).

Carbon Monoxide Hemoglobin. For undetermined reasons, experiments with illuminating gas failed. In one half of the preparations migration of the hemoglobin mass was delayed by 2 mm., compared to oxyhemoglobin A, while in the other half of the runs there was no difference between artificial products and controls. In contrast, carbon monoxide produced by the action of acid on potassium ferrocyanide proved satisfactory. The mobility of carbon monoxide hemoglobin was from 4 to 8 mm. less than that of hemoglobin A. The average delay of a fully saturated sample was 6 mm. Though it was not the same in all preparations, delay was discernable in samples with '25% saturation' and increased with the degree of saturation (Table 1).

Carbon Dioxide Hemoglobin. Differentiation of artificially prepared carbon dioxide hemoglobin from oxyhemoglobin was unsuccessful. Results were erratic. The band was by 2 to 12 mm. faster in two thirds and was slower by 1 to 4 mm. in one third of the runs, as compared to the band for hemoglobin A.

Sulfmethemoglobin. This experiment was a failure. In 4 out of 10 instances there was delay, in 2 there was no change, and in 4 there was acceleration of artificial products, by comparison with hemoglobin A.

Methemoglobin. Results were erratic at all degrees of saturation. In most cases the band was in the same position as that of normal hemoglobin.

Table 1: Mobility of Chemically Altered Hemoglobins and of Normal Hemoglobin

	Saturation %	*Mobility* (*mm.**)	*Delay* (*mm.***)
Cyanhemoglobin	100	61	6
	75	61	6
	50	64	3
	25	64	3
Nitrous Dioxide	100	59	8
	75	61	6
	50	62	5
	25	67	0
Carbonmonoxide	100	62	4
	75	64	3
	50	66	1
	25	66	1
Oxyhemoglobin A	—	67	0

* Under experimental conditions (see *Methods*).

** Compared to normal adult human hemoglobin A.

Discussion

No references to electrophoretic identification of chemically altered hemoglobins were found in the literature. Our attemps met with but limited success. One of the reasons for failure was technical difficulty. The rate of mobility was expressed by the distance of the point of maximum intensity of the stained band from the starting line. The starting and finishing lines were drawn after the strips were stained, to facilitate densitometric scanning. Material for analysis was placed on the filter paper between two pencil marks at the margins of the strip. Differences in migration rates between two samples were sometimes but 1 or 2 mm. Thus, even the slightest variation in the starting point would result in erroneous measurement of the distance. Even the experienced technician would have to set up multiple runs and controls.

Recently, starch electrophoresis at pH 7.2 was used by Gerald, Cook, and Diamond[1] to study a familial atypical methemoglobin M. Attention was again focused by them on the fact that spectroscopic identification of altered hemoglobins is often unsatisfactory and the value of chemical methods is limited. Thus, any method yielding additional information should be welcome. Our failure to separate carbon dioxide hemoglobin, sulfmethemoglobin and neutral methemoglobin is outweighed by successful separation of pure carbon monoxide hemoglobin, cyanhemoglobin and nitrous dioxide hemoglobin, since these 3 varieties are the ones encountered in clinical, industrial and legal medicine.

It was encouraging to learn that migration of these 3 pigments was not the same and that differences occurred not only at full, but also at partial saturation.

Summary

1. Carbon monoxide-, cyan- and nitrous dioxide hemoglobin can be differentiated from oxyhemoglobin by paper electrophoresis. The mobility of chemically altered pigments is slower than that of oxyhemoglobin.

2. Attempts to separate carbon dioxide-, sulf-, and methemoglobin were unsuccessful.

3. The observations may have limited usefulness in clinical and toxicologic investigations.

4. Further experiments, using starch electrophoresis in addition to filter paper, seem desirable.

Part II

The second event was a request by law-enforcing authorities to examine blood stains on 3 garments of 2 persons involved in a case of robbery with assault. Using precipitin and absorption tests, all stains were identified as

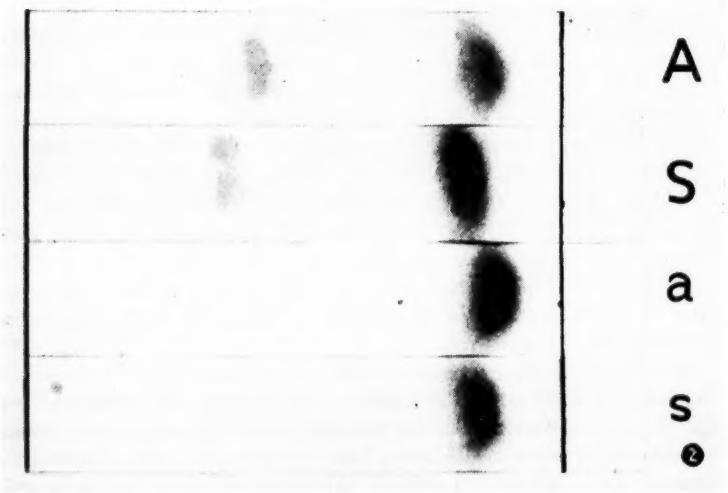

Fig. 2. Elphoregrams of hemoglobin 'A' and 'S' extracted from venous blood and of corresponding blood stain extracts 'a' and 's'.

Mobility of hemoglobin 'A', 76; of 'S', 75; of extract 'a', 79; and of 's', 77 mm. from starting line.

human group ' O ' blood. When it was learned that the assailant, a Negro, had been suspected of having sickle cell anemia a few months before the incident, venous blood from both men and multiple blood stain extracts were processed by paper electrophoresis. Hemoglobin S was found in the Negro's blood. Extracts of stains from one piece of clothing yielded a band located much closer to the position of S hemoglobin than to that of A hemoglobin (Fig. 2). The observation led to the study of artificial blood stains.

Materials and Methods

Blood Stains. Drops of normal adult human blood, of blood from patients with sickle cell anemia, from dogs, cats, rabbits and chickens were placed on pieces of linen or woollen material. The blood stains were allowed to dry at room temperature and placed in envelopes which were sealed, coded and stored in a drawer in the laboratory. Pieces measuring 1 sq. cm. were cut from the material as early as 6 hours and as late as 5 months after the stains were made. The pieces were placed in small test tubes containing 1 ml. of distilled water each. The tubes were stoppered to prevent evaporation and were placed in the ice box for 16 hours. The tubes were centrifuged and 10 lambda of the supernatant fluid were placed on strips of Whatman No. 1 filter paper. Hemoglobin extracted from blood[4] was processed simultaneously. With each run normal human blood, human blood stains and unstained material were used as controls.

Electrophoresis took place in a sodium barbital-sodium acetate buffer of pH 8.6 and ionic strength of 0.1 mho, at 3.2 milliamperes and 110 volts, and at 7–10°C. for 14 hours.

Results

Blood Stains: A and S. Stains from 3 patients with sickle cell anemia were easily distinguished from stains prepared with blood from healthy persons. In one patient with sickle cell anemia a repeat blood sample was obtained after transfusions. The S-band became weaker and moved faster than before and an A-band appeared. These changes were noted in extracts from the stain as in preparation from blood.

Table 2: Mobility of Blood Extracts and Blood Stain Extracts of Various Species

	Blood Extracts		*Stain Extracts*	
Species	*Mobility (mm.*)*	*Variation (mm.**)*	*Mobility (mm.*)*	*Variation (mm.**)*
Rabbit ..	79	+2	78	+2
Human ..	77	0	76	0
Chicken P.R.†	76	—1	74	—2
Chicken W.L.†*	76	—1	73	—3
Dog	75	—2	74	—2
Cat	74	—3	73	—3

* Under experimental conditions (see *Methods*).
** Compared to normal adult human hemoglobin A.
† Plymouth Rock.
†* White Leghorn.

Blood Stains: Various Species. Blood samples from 5 dogs, 5 cats, 5 rabbits and 5 chickens were used for the preparation of stains. With the exception of the chicks, there was satisfactory agreement in the elphoregrams of the hemoglobins and the corresponding stain extracts. There also was uniformity for each species with individual variations staying within limits similar to those for human beings. On the average, rabbit hemoglobin migrated faster by 2 mm. while dog hemoglobin trailed human hemoglobin A by 2 mm. and cat hemoglobin traveled 3 mm. slower than human hemoglobin. Chicken hemoglobin almost paralleled human hemoglobin but trailed it in extracts of stains by either 2 or 3 mm. Aging of stains had no effect on paper electrophoretic patterns (Table 2).

Discussion

Study of blood stains is technically simple. Variation in supporting material might require mechanical squeezing but, by and large, overnight extraction will suffice. One might expect alterations of blood stains in the course of drying or aging. Brown and Kirk,[1] who recently attempted identification of individuals by electrochromatophoretic study of serum proteins of dried blood stains, observed the first effect of time after one year's storage of the

stains. Certainly, one cannot compare storage of material in a drawer to conditions outside a laboratory.

One word of caution, however, seems necessary. Comparison of 2 blood samples is difficult unless both have the same hemoglobin content. Thus, in a run of normal adult blood and blood of a patient with sickle cell anemia the hemoglobin extract of the control blood has to be diluted.

Electrophoresis of hemoglobins of various species has been used rather frequently. Rodnan and Ebauch[2] quote 11 pertinent references; a twelfth paper[3] was published shortly before theirs. No references to hemoglobin extracted from blood stains were found.

The bibliography reveals multiple discrepancies with regard to mobility and number of hemoglobin components. Different results were reported for a certain species by two or more authors. Different results for members of a single species were observed even by the same investigator. Some discrepancies were explained by persistence or continued production of fetal hemoglobin and some by the illness of the animals. Some of the unexplained differences might be due to technic. In our experience, crystals of oxalate did not prevent clotting of chicken blood. We had to use a 2% solution of sodium citrate. At that, samples had to be processed within 4 hours. Human blood used as control was handled accordingly.

No attempt was made to compete in the study of mammalian or avian hemoglobins with those who investigated much larger series.[2, 3] The purpose of our study was to demonstrate parallelism between elphoregrams of hemoglobin extracted from circulating blood and extracts of blood stains of a given sample.

Species identification of blood stains might be feasible if one could obtain samples of blood suspected or alleged to have produced such stains.

Since differentiation of blood stains containing hemoglobin S from those with A was successful, one might expect that identification of other abnormal hemoglobins, namely F, is possible. This part of our study might prove valuable since, in a limited number of instances, it might aid in identification or exclusion of a suspect.

Summary

1. Blood stains of human blood with the hemoglobin S can be separated from stains with hemoglobin A by paper electrophoresis of watery extracts.

2. Blood stains of rabbit, dog, cat and chicken blood can, with proper controls, be differentiated from human blood stains.

3. Provided that blood suspected or alleged to be the source of a stain is available as a control, the observations may have some usefulness in forensic investigations.

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An Investigation of the Distribution of Arsenic by the Sulphide-Silver Method

In Arsenic Poisoning

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This paper is concerned with the investigation of the distribution of arsenic in various organs by the sulphide-silver method[1, 4] in arsenic poisoning.

Preliminary Studies

Arsenic is a semi-metallic substance. In addition to the metalloid modification there is a metallic, yellow arsenic.

Arsenic is precipitated by hydrogen sulphide in acidic solution with the formation of gold yellow arsenic sulphide As_2S_3. In neutral solution colloidal suspensions are liable to form with a negative charge of arsenic sulphide particles. In alkaline sulphide, ammonium sulphide and sodium hydroxide arsenic (III) sulphide dissolves with the formation of oxothio-complex ions. On acidification the arsenic sulphide is re-precipitated.

It was first investigated whether the arsenic sulphide acts as a catalyser in the physical development, i.e. whether it can be demonstrated by the accumulation of metallic silver round the arsenic sulphide particles.

Experiment. Slides fixed in alcohol were placed for 24 hours in an aqueous arsenic solution at 50° C. and afterwards treated with hydrogen sulphide solution without previous rinsing. The slides were then washed repeatedly in distilled water and alcohol and afterwards placed in the physical (citric acid) developer.

Results. The yellow deposits in the sections soon turned black, thereby showing that the arsenic sulphide acts as a catalyser in the process of development.

Investigation Proper

Material

Human. The internal organs of a 49-year-old woman, who had died from arsenic poisoning, were used. Chemical analysis had shown the following arsenic values:

Liver, 8 mg. As per 100 g.

Kidneys 5 mg. per 100 g.

Blood 0.7 mg. per 100 g.

(Dr. Arne Dyfverman, Statens Rättskemiska Laboratorium, Stockholm.)

Animal. The material consisted of: Internal organs from one hamster given arsenic in a dose of about 500 mg. per 100 g. body weight *per os* and which had died within 2 hours.

Internal organs from one hamster and one rat that had received 0.01 mg. arsenic per 100 g. body weight subcutaneously in aqueous solution twice a week for one month.

Method

Parts of the liver, kidneys and pancreas were fixed in ammoniacal, neutral and in acidified (formic acid, pH 3–4) alcohol saturated with hydrogen sulphide. The sections were then treated with increasing concentrations of alcohol with or without ammonia and with or without formic acid.

The de-paraffinized sections were placed in the physical developer.

Results in Man

Kidneys. In material fixed in 'neutral' or acid solutions the walls of the glomerular capillaries showed fine granules. In addition, especially in the lumina of the proximal convoluted tubules and the luminal portion of the cytoplasm of the epithelial cells, there was dense granulation. Such granulation was also seen in the thin and the thick limbs of the loop of Henle. In the thin limbs and in the distal convoluted tubules, the granules were less crowded. Moreover, fine black granulation was readily recognized in the walls of the capillaries between the proximal convoluted tubules (Fig. 1).

In the material fixed in the ammoniacal fluid only faint or no black granulation was seen in segments of the tubules in the periphery of the

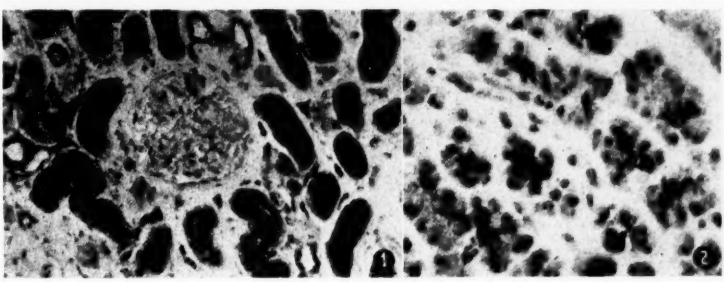

Fig. 1. Human arsenic poisoning (kidney). Sulphide-silver method, acid fixation. Paracarmine

Fig. 2. Arsenic poisoning. Excorine part of pancreas. Sulphide-silver method, acid fixation. Paracarmine.

sections. In the middle of the sections the picture was, however, the same as in sections fixed in 'neutral' or acid media.

Liver. In material fixed in the acid or in the 'neutral' solution, dense black granules were seen, usually centrally, in the cytoplasm of the parenchyma cells. In the centres of acini the granulation was more dense than in the periphery of the sections.

Pancreas. In material fixed in the acid or 'neutral' solution the lumina of the tubules were densely packed with black granules (Fig. 2); the island cells were also blackened, though less intensely in the material fixed in the acid solution.

In material fixed in the ammoniacal solution black granulation appeared in all the islands cells but not in the exocrine tissue.

Animal Material

Acute Poisoning (*Hamster*)

Kidneys fixed in acid or 'neutral' solution showed fine black granulation in some of the glomeruli. It was not possible to say where in the glomeruli the granules were situated. Black granules were also sometimes seen in Bowman's capsule. The lumina and the luminal part of the cytoplasm of the epithelial cells of the proximal convoluted tubules showed dense black granulation. In specimens fixed in alkaline medium only a few scattered granules were seen, and then in the epithelium of the distal convoluted tubules.

Liver fixed in acid or 'neutral' medium showed the following features: In the centres of the acini parenchymal cells contained granules located in the middle of the cytoplasm. Granules were also seen in the Kupffer cells.

In material fixed in alkaline fluid no granules could be demonstrated.

Pancreas. The islands of Langerhans showed distinct black granulation, which was not so pronounced in specimens fixed in acid solution. In these sections the granules appeared to have clumped rather than to have arranged themselves according to the morphological structure of the tissue. In the exocrine part, the lumina of the tubules and of the ducts contained crowded black granules.

In material fixed in alkaline medium only the islands of Langerhans appeared black. The exocrine part contained no granules.

Chronic Poisoning

Rat: Kidneys. In preparations fixed in acid or 'neutral' solution the glomeruli, especially the walls of the glomerular capillaries, contained black granules. The proximal convoluted tubules showed dense black granula-

tion in the lumina and in the luminal part of the cytoplasm of the epithelial cells.

In material fixed in alkaline solution only single granules were seen in the epithelial cells of the proximal convoluted tubules.

Liver. In material fixed in acid or 'neutral' solution black granules were seen in the cytoplasm of the parenchyma cells in the centres of the acini.

No granules were detectable in the material fixed in alkaline solution.

Pancreas. The islands of Langerhans showed the usual picture.[4] Black granules were seen in the lumina of the tubules and of the ducts.

Preparations fixed in alkaline solution showed only the usual granulation of the islands.

Hamster: The findings were the same as in the rat, except that the granulation in all the island cells was equally dense.

Discussion

The preliminary experiments showed that arsenic sulphide acts as a catalyser. It accelerates the reaction reduction-oxidation process between the silver ions and a reducing agent (hydroquinone). As pointed out above, precipitation of arsenic as arsenic sulphide is optimal in acid solution but also possible in neutral solution.

However, as is apparent from the results of the experiments, in human material a positive result was also obtained when the tissue was fixed in alkaline (ammoniacal) alcohol saturated with hydrogen sulphide. The chemistry of this reaction is difficult to explain. It should, however, be recollected that post-mortem changes and fixation are accompanied by some, though slight, acidification of the material.[5, 6] This is most pronounced on fixation in formaldehyde and least on fixation in alcohol. Therefore, even when the specimen is fixed in an alkaline medium, a certain acidification of the tissue, especially of the tissue situated deeper in the specimen, cannot be excluded.

This assumption is strengthened by the observation that the granulation was less pronounced in the peripheral than in the central parts of the sections. That this difference was not seen in animal material may be explained by the fact that the specimens studied were smaller and were fixed immediately after the death of the animals.

As shown in earlier investigations, black granules can be demonstrated in the luminal part of the cytoplasm of the epithelial cell of the distal convoluted tubules of the kidneys in normal human beings and in the epithelium of the proximal convoluted tubules in the hamster. Granules sometimes appear in the centres of acini in the liver and always abundantly in the islands of Langerhans. In the normal rat the peripheral cells in the islands of Langerhans were blacker than the central cells.[4]

All the other silver granules seen in *arsenic poisoning* in the present investigation indicate the substance precipitated by the hydrogen sulphide and referable to the arsenic poisoning.

Provided that the method really demonstrates arsenic, and the reactions in acid and alkaline solutions suggest that it does, the arsenic appears in the glomerulus and is then demonstrable in the proximal convoluted tubules. In the human kidney accumulations of arsenic also appeared in the walls of the capillaries between the proximal convoluted tubules.

In the liver the arsenic was seen mainly in the centres of the acini and only little was found in the Kupffer cells.

Judging by the literature, it was not known that arsenic tends to accumulate in the pancreas and to be excreted with the pancreatic juice.

In the event of metal poisoning the pancreas appears to fulfil an important function as an outlet, it also having been shown histochemically, for example, that in cobalt poisoning[2] and in zinc poisoning[4] the metal can be excreted via the pancreas. It is thus clear that the pancreas is useful as a specimen for chemical investigation of cases of suspected arsenic poisoning.

The similarity between the appearance of the sections of material from man, the rat and the hamster was striking.

The sulphide-silver method cannot, of course, replace ordinary methods for the investigation of arsenic poisoning, but it does map out the sites of the substances precipitated by hydrogen sulphide.

Summary

The sulphide-silver method for the histochemical demonstration of heavy metals is also useful for demonstrating arsenic.

The best results are obtained when the tissue is fixed in acidified (formic acid) alcohol saturated with hydrogen sulphide.

In arsenic poisoning the arsenic appears in the renal glomeruli and in the convoluted tubules in animals and in Man; in the latter also in the walls of the capillaries.

In animals as well as in Man arsenic was also found in the plasma of the parenchyma cells in the centres of the liver acini and in Kupffer cells. Arsenic appears in the exocrine part of the pancreas (gland tubules and duct).

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